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Preventive Veterinary Medicine 57 (2003) 127–139

PREVENTIVE VETERINARY MEDICINE

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Factor analysis of minimum-inhibitory concentrations for *Escherichia coli* isolated from feedlot cattle to model relationships among antimicrobial-resistance outcomes

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Received 18 April 2002; accepted 29 October 2002

Abstract

Factor analysis was used to assess relationships in the minimum-inhibitory concentration among 17 antimicrobials tested on isolates of *Escherichia coli* isolated from 360 faecal samples obtained from feedlot cattle. Six factors were extracted using maximum-likelihood factor analysis. The factors were interpretable antimicrobial groupings based on class of antimicrobial and previously described associations. New-generation cephalosporins, older-generation beta-lactams, fluoroquinolones and aminoglycosides grouped separately as classes of antimicrobials on four of the six factors. One of the remaining factors was a grouping of antimicrobials that had been identified as being related in previous feedlot studies. The last factor was a grouping of three of the five antimicrobials that comprise the antimicrobials found in penta-resistant strains of *Salmonella* Typhimurium. The factor analysis described patterns in the MIC data that would not have been apparent if only antimicrobial-resistance data categorized as susceptible-resistance had been analysed.
Published by Elsevier Science B.V.

Keywords: Antimicrobial resistance; Factor analysis; Minimum-inhibitory concentration; *Escherichia coli*; Feedlot cattle; Faecal samples

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0167-5877/02/$ – see front matter. Published by Elsevier Science B.V.
doi:10.1016/S0167-5877(02)00232-5

1. Introduction

Antimicrobial resistance, especially in pathogenic bacteria, is a global problem that has emerged in the past two decades (Blondeau and Vaughan, 2000; Aarestrup, 2000). Resistance in bacterial isolates from agricultural animals has raised concern both among public-health professionals and veterinarians. The public-health concern is that antimicrobial-resistance genes can be incorporated into the genome of potential human pathogens (e.g. *Salmonella*, *Campylobacter*, *Escherichia coli*) and transmitted through food-borne routes to humans. In addition, there is concern that resistance genes in non-pathogenic bacteria could be transmitted to humans where the genes would be available to non-resistant pathogenic bacteria in the human gastrointestinal tract. Veterinarians and other animal-health authorities are concerned about their ability to control bacterial infections in domestic animals and about the zoonotic potential. These concerns have motivated considerable effort in both human medical and veterinary areas to identify emerging resistance, estimate prevalence, and monitor trends (Sahm et al., 2001; Jones, 2000; Cavallo et al., 2000; Smith et al., 1999).

The importance of resistance-surveillance mandates the development of effective and efficient monitoring systems (Sahm et al., 2001). Within the framework of surveillance, there is a need to refine statistical methods for identifying patterns of resistance and to be able to communicate the results to the scientific community (Jones, 2000). Surveillance systems can generate large amounts of data that must be distilled into meaningful summaries. An important attribute of resistance-surveillance data is the occurrence of multiple outcomes per individual bacterial isolate. Multiple outcomes typically arise when a panel of antimicrobials is tested on each isolate. Methods that have been used to address the multiple outcomes include antibiograms, indices (Krumperman, 1983; Kaspar et al., 1990), and discriminant analysis (Wiggins, 1996).

Antibiograms are essentially a tabulation of the occurrence of antimicrobial resistance. Resistance to a specific antimicrobial can occur singly or jointly with other antimicrobials. Interpretation of antibiograms depends on visual examination of the tabulation to identify patterns. Indices typically are a summary value of the richness, the number of antimicrobials to which an isolate is resistant, or diversity of the pattern being examined. The summary value yields little information concerning the patterns of resistance in the data. Discriminant analysis is the only method that addresses the multiple outcomes with multivariate statistical methods. The objective of the discriminant analysis was to construct a linear function of resistant/non-resistant responses to multiple antimicrobials to separate isolates into categories for identification of sources such as in the case of non-point source pollution (Wiggins, 1996).

Our objective was to describe the use of factor analysis in identification of patterns in antimicrobial minimum-inhibitory concentrations (MICs). Specifically, factor analysis was applied to identify patterns within a panel of 17 antimicrobials used to test bacterial isolates of *E. coli* from faeces of feedlot cattle. The usefulness of the method was assessed by the ability to identify patterns of both susceptibility and resistance that had interpretations based on the biology of resistance development and antimicrobial action.

2. Materials and methods

2.1. Study population

Faecal samples were collected from cattle housed in six pens at a commercial feedlot in Colorado on 2 May 2000. Each pen had approximately 10,000 square feet of area and contained between 54 and 56 beef steers. Cattle in three of the pens had been on feed for 75 days while the other three pens had been on feed for 81 days. All cattle were receiving tylosin in their rations at a rate of 10 g/t of feed (approximately 60–90 mg per head per day). One animal had received an injection of an antimicrobial (micotil) in the week prior to sampling. No other cattle had antimicrobial injections in the month prior to sampling. Within each pen, 30 fresh faecal samples (approximately 50 g each) were collected from individually identifiable faecal pats on the ground. Disposable plastic gloves were used to pick up faecal material and were changed between samples. Efforts were made to collect the faecal samples from sites throughout the pen to minimize the opportunity for collecting multiple faecal samples from the same animal.

Additionally, while the pen floor samples were being collected, 30 faecal samples were obtained per rectum from randomly selected individual animals from each of the six pens while the cattle were being restrained in a chute. The randomization was performed by assigning random numbers to the count of cattle in the pen (numbers ranging from 1 to 54 or 56 depending on pen size). The random numbers were sorted and the top 30 were chosen for sampling. The count value was translated to the order going through the chute. All samples were cooled and transported to the laboratory for further processing within 4 h of collection.

In the laboratory, approximately 1 g of faecal material from each sample was placed in a 50 ml conical tube. Ten millilitre of 0.5% normal saline was added to each tube. The tubes were shaken using a vortex to mix the saline and the faecal material. The tubes were then kept overnight in a refrigerator at 3 °C. On the next day, the samples were shipped for overnight delivery to a second lab where the culturing and antimicrobial susceptibility testing was conducted.

2.2. Culture and antimicrobial-resistance testing

Dilute faecal material was streaked on MacConkey-4-methylumbelliferyl-β-D-glucuronide agar plates agar plates to isolate colonies of *E. coli*. *E. coli* was chosen as the species for antimicrobial-resistance testing because of its ubiquitous nature. Plates were incubated at 37 °C for 18–24 h. Individual *E. coli* colonies were identified under ultraviolet light as lactose-positive (bright pink) and glucouronidase-positive (colony periphery had a bluish appearance). Five colonies were selected from each agar plate and transferred to individual nutrient agar slants and incubated for another 18–24 h. The slants then were checked for growth and stored at 2–8 °C until the individual isolates were tested for susceptibility. In preparation for antimicrobial susceptibility testing, MacConkey agar plates were inoculated with bacteria from the slants. Colonies were selected from these plates and placed into separate tubes with 5 ml of sterile water and turbidity was adjusted to a 0.5 McFarland standard. After mixing, 10 μl of the bacterial suspension was used to inoculate 10 ml of

Table 1
Descriptive statistics for MIC data ($n = 1737$ *E. coli* isolates from 360 feedlot cattle faecal samples tested for drug, resistance against *E. coli*) (MIC units are μg/ml)

Antimicrobial	5th percentile	Median	95th percentile	Maximum	Non-transformed skewness	Transformed skewness
Amikacin	4.0	4.0	4.0	16.0	23.6	18.4
Amoxicillin–clavulanic acid	1.0	4.0	4.0	32.0	7.8	−0.1
Ampicillin	2.0	2.0	4.0	32.0	6.7	2.8
Apramycin	2.0	4.0	4.0	16.0	2.3	0.0
Cefoxitin	4.0	4.0	4.0	32.0	11.9	7.7
Ceftiofur	0.5	0.5	0.5	4.0	14.8	13.3
Ceftriaxone	0.25	0.25	0.25	8.0	18.2	14.4
Cephalothin	2.0	4.0	16.0	32.0	3.1	0.0
Chloramphenicol	4.0	8.0	8.0	32.0	4.5	1.0
Ciprofloxacin	0.015	0.015	0.015	0.025	14.4	8.3
Gentamicin	0.25	0.50	0.50	4.0	5.7	0.5
Kanamycin	16.0	16.0	16.0	64.0	9.7	9.5
Nalidixic acid	4.0	4.0	4.0	128.0	12.8	9.5
Streptomycin	32.0	32.0	64.0	256.0	6.6	3.9
Sulphamethoxazole	128.0	128.0	512.0	512.0	1.3	1.3
Tetracycline	4.0	4.0	32.0	32.0	1.0	0.9
Trimethoprim	0.12	0.12	0.25	4.0	16.2	3.2

cation-adjusted Mueller-Hinton broth, which then was used to inoculate antimicrobial-sensitivity plates. The plates were incubated at 35 °C for 18–24 h and then read using a semi-automated system (Sensititre™). Based on growth in individual wells, the MICs against a panel of 17 antimicrobial drugs were determined. The 17 antimicrobials (Table 1) were selected to parallel the panel used by the National Antimicrobial Resistance Monitoring System for Enteric Bacteria (NARMS-EB) in the US. The ATTC *E. coli* 25922 was used as a quality control organism. The MIC for each antimicrobial was used to classify the isolate as susceptible, intermediate, or resistant according to standards used to classify human isolates as determined by the National Committee on Clinical Laboratory Standards. (Breakpoints for veterinary MICs values are not currently available.)

2.3. Statistical analysis

All analyses were performed using Version 8.1 of SAS.[1] All observations with missing MIC results for any of the 17 antimicrobials were deleted for all analyses. Descriptive statistics were computed to examine the univariate MIC distributions for the 17 antimicrobials. Some of the techniques we used assume a multivariate normal distribution. To accommodate this assumption, data for individual variables should be approximately normally distributed. Skewness of the MIC distributions indicated the need for a transformation to more-closely approximate normal distributions. The data were log transformed to

[1] SAS Version 8.1: SAS Software, SAS Institute Inc., SAS Campus Drive, Cary, NC 27513.

correct for the lack of normality. Shapiro–Wilk tests for normality were used to assess the normality assumption of the transformed data (Conover, 1980).

Three factor-analytic methods were implemented to allow for comparison of fit criteria: principal-component (PC), unweighted least-squares (ULS), and maximum-likelihood (ML). A varimax rotation was used in each method. The fit criteria examined were root mean square (RMS) of the off-diagonal residual correlation and RMS of the off-diagonal partial correlations. The number of factors to keep in the factor analysis was determined by evaluating three criteria based on results from the PC and ML methods: a scree plot, a minimum eigenvalue of 1.00, and a large-sample test for the number of common factors (Johnson and Wichern, 1992). The scree plot, a plot of the factor number and its associated eigenvalue, was assessed by looking for a levelling of the slope of the curve. The large-sample test was used by sequentially fitting models with increasing numbers of factors kept. A χ^2-test tested significance of adding factors using output from the ML factor analysis. Johnson and Wichern (1992) warn that large-sample sizes and a small number of factors being examined compared to the number of variables entering the factor analysis can lead to retention of more common factors than are necessary to explore the data. Consequently, they suggest exercising judgement in selecting the number of factors since additional factors can be significant without providing much additional insight into data. The scree plot and the minimum eigenvalue cutoff of 1.00 came from the PC method while the χ^2-tests were obtained from the ML method. The final model determined from transformed data was compared to a model developed from non-transformed data to assess the effect of the transformation on the factors. A P-value ≤ 0.05 was used in determining statistical significance (Fig. 1).

Multiple isolates were obtained from a single faecal sample and, potentially, faecal samples could have originated from the same individual making the assumption of independence needed for inferential analysis problematic. We ran the factor model with randomly selected single isolates from each sample to assess the impact that five isolates from a single sample might have had on the model. Johnson and Wichern (1992) discuss an example where the lack of independence among repeated observations was evaluated in this manner.

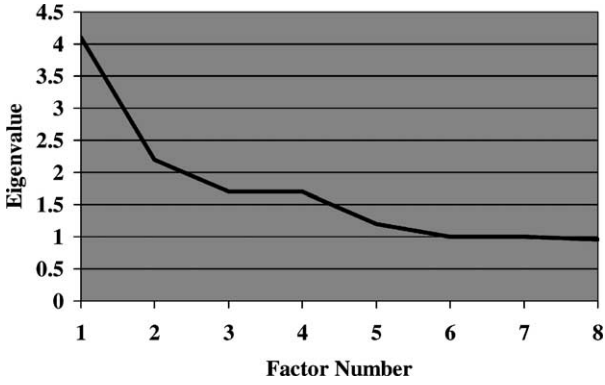

Fig. 1. Scree plot depicting the eigenvalues for eight factors. Factors with eigenvalues of at least one were kept in the final factor model. (Factor analysis model of antimicrobial resistance of 1737 *E. coli* isolates from 360 feedlot cattle faecal samples.)

An antibiogram table was developed for comparison with the factor-analysis results. Intermediate results were combined with susceptible results to create a dichotomous susceptible/resistant outcome for constructing the antibiogram.

3. Results

MIC was measured on a total of 1737 isolates (one isolate lost to testing and 42 isolates with missing MIC data). The descriptive analysis of the untransformed data indicated that the MIC values were not normally distributed (Table 1). MIC distributions for almost all of the antimicrobials were highly skewed and all 17 of the Shapiro–Wilks tests for normality were highly significant ($P < 0.001$). The log transformation improved the skewness problem for all 17 antimicrobials although the Shapiro–Wilks tests for normality still indicated significant deviations from normality ($P < 0.001$) for the transformed MIC values for all antimicrobials. The deviations from normality are likely due to the discrete characteristics of the MIC data and the relatively large-sample size which allows for detection of small deviations from normality.

The antibiogram revealed 36 different combinations of resistance to antimicrobials (Table 2). Most of the isolates (1148/1737; 66.1%) were susceptible to all antimicrobials; resistance to a single antimicrobial was indicated in 189 (10.9%) of the isolates. No resistance was ever observed for six antimicrobials (amikacin, apramycin, ceftiofur, ceftriaxone, ciprofloxacin, and gentamicin). Most of the antimicrobial-resistance combinations were relatively rare (<1.0% of isolates). Varying combinations of resistance to streptomycin, sulphamethoxazole, tetracycline, and trimethoprim–sulphamethoxazole predominated.

Based on the scree plot, five to six factors would be recommended although most of the variation in the data was represented in three to four factors. Based on the criteria of keeping eigenvalues ≥ 1.0, six factors should be kept. The large-sample χ^2-tests used to check for sequential addition of factors indicated that at least seven factors could be kept in the analysis (Table 3). We chose to keep six factors.

The best fit between the six-factor models was judged by examining RMS of the off-diagonal residual correlation and RMS of the off-diagonal partial correlations (Table 4). These two criteria are used to compare the relative ability of the models to fit the observed correlation matrix (1992). Larger values for both the criteria imply a poorer fit because more of the original correlation matrix is left unexplained by the factor analysis. The PC method had the poorest fit based on these two diagnostics. The other two methods had comparable values for the two diagnostics.

The loadings for the six factors nevertheless were examined for all three models. The factor loadings were very similar between the ULS and the ML methods and, thus, only the ML and PC method loadings are presented here (Table 5). The PC loadings differed from the results for the other two methods primarily by a switching of factor 1 and factor 2. Also, kanamycin loaded on the sixth factor extracted using the PC method but did not load on any factor using the other methods. Because of fit criteria, the consistencies between the ULS and ML factor loading patterns, and the general agreement across all three methods, the remaining discussion of results will focus on the factor-analysis results based on the ML method.

Table 2
Frequency distribution of resistance groupings ($n = 1737$ outcomes of *E. coli* isolates from 360 feedlot cattle faecal samples)[a]

Antimicrobials	Frequency	Percent
Amo, Amp, Cef, Cep, Chl, Str, Sul, Tet	6	0.35
Amo, Amp, Cep, Chl, Str, Sul, Tet	1	0.06
Amo, Amp, Cep, Str, Sul, Tet	1	0.06
Amo, Amp, Cep, Tet	1	0.06
Amo, Amp, Cep	3	0.17
Amo, Cep	1	0.06
Amp, Cep, Tet	2	0.12
Amp, Chl, Str, Sul, Tet	10	0.58
Amp, Chl, Str, Sul	2	0.12
Amp, Str, Sul	1	0.06
Amp, Sul, Tet	1	0.06
Amp, Sul	1	0.06
Cef, Sul, Tet	1	0.06
Cef	1	0.06
Chl, Str, Sul, Tet, Tri	2	0.12
Chl, Str, Sul, Tet	5	0.29
Chl, Sul, Tet	1	0.06
Chl, Sul	1	0.06
Chl	2	0.12
Kan, Str, Sul, Tet	8	0.46
Kan, Str, Tet	2	0.12
Kan, Sul, Tet	3	0.17
Kan, Sul	2	0.12
Kan	2	0.12
Nal, Str, Sul, Tet	2	0.12
Nal, Str, Tet	1	0.06
Nal, Sul, Tet	11	0.63
Nal	3	0.17
Str, Sul, Tet	140	8.05
Str, Sul	1	0.06
Str, Tet	15	0.86
Str	4	0.23
Sul, Tet	175	10.07
Sul	26	1.50
Tet	151	8.69

[a] Amo: amoxicillin–clavulanic acid, Amp: ampicillin, Cef: cefoxitin, Cep: cephalothin, Chl: chloramphenicol, Kan: kanamycin, Nal: naladixic acid, Str: streptomycin, Sul: sulphamethoxazole, Tet: tetracycline, Tri: trimethoprim–sulphamethoxazole.

Two antimicrobials, ceftiofur and ceftriaxone, loaded very high (loading > 0.90) on the first factor while a third antimicrobial, cefoxitin, loaded moderately high (loading > 0.50). All three of these antimicrobials are cephalosporins (a type of beta-lactam), both second and third generation. Thus, factor one can be described as ''newer-generation cephalosporins.'' Sulphamethoxazole and tetracycline loaded heavily on factor two while trimethoprim–sulphamethoxazole and, to a lesser extent, streptomycin loaded moderately high. These antimicrobials do not come from a single class of antimicrobials—but resistances to

Table 3
Large-sample χ^2-tests for the number of factors to keep in ML factor-analysis model

Factor to be added	χ^2	d.f.	*P*-value
2	3348.4	16	<0.0001
3	1647.0	15	<0.0001
4	1212.8	14	<0.0001
5	717.5	13	<0.0001
6	396.1	12	<0.0001
7	77.5	11	<0.0001

these two drugs occur together frequently in bacterial isolates. Factor two also can be considered to be a group of antimicrobials with higher prevalences of resistance than other antimicrobials on the panel. Factor three was dominated by amoxicillin–clavulanic acid and cephalothin with a lesser influence by ampicillin. All three of these antimicrobials are older-generation beta-lactams. Only ciprofloxacin and nalidixic acid loaded high on factor four. These are the only fluoroquinolones in the panel. Consequently, factor four is a grouping of fluoroquinolones. Gentamicin loaded high while apramycin loaded moderately high on factor 5. Both antimicrobials are in the aminoglycoside class of antimicrobials. Other aminoglycosides (specifically amikacin, kanamycin, and streptomycin) did not load high on factor 5 (amikacin and kanamycin actually had negative loadings on factor 5). Ampicillin, chloramphenicol, and streptomycin represent the highest loadings on factor 6. Chloramphenicol had its highest loading on factor 6, while the loadings in factor 6 for ampicillin and streptomycin were similar to those in factor 3 and factor 2, respectively.

Neither amikacin nor kanamycin (both aminoglycosides) loaded strongly on any of the factors created using either the ML or the ULS methods. Amikacin dominated the sixth factor created by the PC method.

The ML method was run to extract six factors from the untransformed data. The resulting loading patterns were the same as obtained from the transformed data although the order of the factors was mixed and streptomycin and trimethoprim–sulphamethoxazole did not load as highly with sulphamethoxazole and tetracycline (data not shown).

ML models using a randomly selected single isolate consistently mirrored the results that we obtained when all five isolates were available to the model (data not shown). However, because of the reduced number of isolates in the single isolate model, occasionally the model would not solve because of singularities in the model (all MIC values the same for at least one antimicrobial).

Table 4
RMS for the off-diagonal residual and partial correlations for the six-factor models of antimicrobial resistance of *E. coli* isolates from 360 feedlot cattle faecal samples

Factor-analysis method	Residual RMS	Partial correlation RMS
ML	0.01	0.03
PCs	0.06	0.21
ULS	0.01	0.03

Table 5
Loadings[a] for the six factors from the ML and PC factor analysis of MICs for *E. coli* isolates from 360 feedlot cattle faecal samples

Antimicrobial	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6
ML loadings						
Amikacin	−0.010	0.039	0.033	0.007	−0.041	0.011
Amoxicillin–clavulanic acid	0.166	0.088	*0.769*	0.023	0.120	0.192
Ampicillin	0.196	0.092	*0.470*	0.025	0.064	*0.557*
Apramycin	0.017	0.070	0.138	0.015	*0.648*	0.098
Cefoxitin	*0.552*	0.015	0.252	−0.025	−0.007	0.192
Ceftiofur	*0.955*	0.056	0.082	0.016	0.031	0.133
Ceftriaxone	*0.949*	0.049	0.053	0.030	0.028	0.137
Cephalothin	0.125	0.152	*0.807*	0.036	0.124	0.029
Chloramphenicol	0.148	0.053	0.074	−0.028	0.039	*0.486*
Ciprofloxacin	0.024	0.034	0.035	*0.893*	0.042	−0.026
Gentamicin	−0.007	0.047	0.115	0.014	*0.891*	0.049
Kanamycin	−0.016	0.125	0.000	−0.006	−0.019	0.009
Nalidixic acid	−0.008	0.037	0.024	*0.776*	−0.026	−0.006
Streptomycin	0.266	*0.537*	0.021	−0.012	0.146	*0.495*
Sulphamethoxazole	0.068	*0.891*	0.081	0.111	0.066	0.031
Tetracycline	0.055	*0.809*	0.170	0.110	0.074	0.011
Trimethoprim–sulphamethoxazole	0.084	*0.695*	0.083	−0.061	0.058	0.097
PC method						
Amikacin	0.034	−0.061	0.107	0.002	−0.135	0.135
Amoxicillin–clavulanic acid	0.100	0.156	*0.863*	0.020	0.114	0.029
Ampicillin	0.087	0.294	*0.637*	0.007	0.093	*0.341*
Apramycin	0.084	0.014	0.135	0.011	*0.869*	0.007
Cefoxitin	0.025	*0.715*	0.296	−0.036	−0.024	0.006
Ceftiofur	0.099	*0.938*	0.081	0.022	0.032	0.015
Ceftriaxone	0.093	*0.940*	0.053	0.034	0.028	0.020
Cephalothin	0.170	0.082	*0.857*	0.038	0.085	−0.065
Chloramphenicol	−0.024	0.244	0.139	−0.055	0.116	*0.752*
Ciprofloxacin	0.041	0.019	0.029	*0.918*	0.043	0.005
Gentamicin	0.083	−0.023	0.130	0.011	*0.873*	−0.006
Kanamycin	0.103	−0.120	−0.049	0.028	−0.099	*0.548*
Nalidixic acid	0.039	−0.002	0.022	*0.914*	−0.023	−0.019
Streptomycin	*0.608*	0.346	0.064	−0.036	0.183	*0.402*
Sulphamethoxazole	*0.892*	0.021	0.078	0.114	0.034	0.089
Tetracycline	*0.852*	0.014	0.165	0.116	0.031	0.025
Trimethoprim–sulphamethoxazole	*0.825*	0.072	0.083	−0.103	0.026	−0.023

[a] Moderate and high loadings are in italics.

4. Discussion

Patterns in MICs identified by factor analysis essentially fell into two types of relationships: a link amongst antimicrobials within the same class (e.g. cephalosporins, fluoroquinolones, or aminoglycosides) and groupings of antimicrobials (not necessarily from the same class) that were consistent with susceptibility/resistance associations in other studies of antimicrobial resistance in beef cattle.

The ability to attach specific interpretations to the factors demonstrates the value of this type of analysis. Factor one is a grouping of newer-generation cephalosporins. Under selection pressure for the development of resistance for at least one new-generation cephalosporin, it is logical that resistance might emerge for all the cephalosporins. Similarly, in the absence of selection pressure for this class of antimicrobials it is also logical to find them loading together on the same factor. Thus, factor one represents a susceptibility pattern in the data that might not be apparent when reviewing the antibiogram (which is based solely on resistance).

Factor two differs from factor one in that the former represents a mixture of antimicrobial classes. Three of the four antimicrobials in this group had higher univariate MIC values (Table 1) and the highest frequency of resistance (Table 2) among the antimicrobials tested. However, high MIC values and frequency of resistance does not appear to be the reason that trimethoprim/sulphamethoxazole was correlated with the other antimicrobials. Other studies of resistance patterns of enteric bacteria from feedlot cattle (Dargatz et al., 2002) and beef cows (Dargatz et al., 2000) have demonstrated the co-occurrence of streptomycin and trimethoprim/sulphamethoxazole resistance as well as sulphamethoxazole and tetracycline resistance.

The third factor was comprised of antimicrobials from the older group of beta-lactams. As with factor one, this grouping appears to be a grouping of antimicrobials from the same class. One difference between the antimicrobials that loaded high in factor 3 and factor 1, respectively, is that the MIC values tend to be higher in the antimicrobials with higher loadings on the factor 3. In factor 3, the antimicrobials with high loadings have been in use for a longer period of time and, perhaps, are more-frequently used in feedlot operations. Approximately 50% of large feedlots ($\geq$8000 head) use cephalosporins (ceftiofur) to treat respiratory disease (USDA, 2000)—one of the most-common diseases in feedlot cattle. However, only about 9% of feedlots use it as their primary initial treatment (USDA, 2000)—so this class of antimicrobials is being used only in special circumstances as a primary treatment. Treatment records from the study feedlot indicated beta-lactams were not used as a primary initial treatment for respiratory disease.

Factor 4 was dominated by the fluoroquinolones, a new class of antimicrobials of special concern because resistance has not yet developed widely in the human or animal populations (Sahm et al., 2001; Smith et al., 1999; Thornsberry et al., 1999). Nalidixic acid, the older of the two fluoroquinolones, had relatively higher MIC values which were reflected in the presence of resistance in some isolates but ciprofloxacin resistance was absent in these 1737 isolates. Consequently, the factor analysis was able to identify a correlation between the two fluoroquinolones using MIC data that would not have been apparent using SIR data. Ruiz et al. (Ruiz et al., 2002) demonstrated a relationship between naladixic acid resistance prevalence and ciprofloxacin MIC values in human *E. coli* isolates. In their study, increasing prevalence of nalidixic acid resistance was associated with increasing ciprofloxacin MIC values in ciprofloxacin-susceptible isolates.

Factor 5 is problematic because only two of the five aminoglycosides, apramycin and gentamicin, loaded high on the factor (amikacin, kanamycin, and streptomycin did not). Streptomycin is an older antimicrobial and its common link with other antimicrobials was strongly represented in factor 2. Amikacin is a synthetic derivative of kanamycin so its activity against bacteria is similar (Prescott et al., 2000). Consequently, it is not surprising

to see the two behave similarly in a factor analysis. However, the relationship, or lack thereof, between these two antimicrobials and the two that loaded heavily on this factor is not well understood.

The antimicrobials in factor 6, in the same manner as factor 2, come from different classes of antimicrobials. The grouping of antimicrobials in factor 6 parallels an important combination of antimicrobial resistance found in *Salmonella* Typhimurium. An epidemic strain of *S.* Typhimurium definitive type 104 has a penta-resistant combination to ampicillin, chloramphenicol, streptomycin, sulphonomides and tetracyclines and a world-wide distribution (Davis et al., 1999). The latter two antimicrobials did not load substantially on factor 6 but loaded heavily on factor 2.

Six antimicrobials (amikacin, apramycin, ceftiofur, ceftriaxone, ciprofloxacin, and gentamicin) did not contribute information to the antibiogram because all isolates were susceptible. When the MIC values were used in the factor analysis, only amikacin was not related substantially to any of the six factors. The categorization of MIC values into susceptibility/resistance categories for creation of the antibiogram resulted in a loss of information.

Many reports present only the dichotomy of susceptibility/resistance for each antimicrobial on the panel that was tested in the study along with the breakpoint for designation (Walker and Thornsberry, 1998). Solely reporting descriptive information univariably on resistance/susceptibility has some disadvantages. First, the MIC breakpoints could change—thereby limiting the value over time of studies, which dichotomized the data (Walker and Thornsberry, 1998). Secondly, dichotomization reduces the amount of information that is available for analysis and interpretation. Shifts in MIC values might become apparent or be of interest before ''cutpoints'' are crossed. Jones (2000) presents a case for showing the MICs as cumulative distributions. Lastly, the biological processes underlying resistance development and transmission lead to resistance/susceptibility linkages among antimicrobials that are not easily identifiable when univariate summaries are presented.

The potential for violation of assumptions when using MIC data in factor analysis should be assessed. The MIC data are not truly continuous data and might even be left or right truncated. These characteristics—along with the potential for skewness in the distributions—make it difficult to assume that the underlying distribution is normal. The log transformation we used in this study removed only some of the skewness. These deficiencies make the assumption of multivariate normality somewhat tenuous unless, with the large-sample size in the current study, the multivariate version of the Central-Limit Theorem is invoked (Morrison, 1976). The similarity of the factors obtained using the PC and the ULS methods—neither of which depend on multivariate normality for factor extraction (Johnson and Wichern, 1992; Harman and Jones, 1966)—with the factors obtained using the ML method suggests that the ML method provided valid results even if the multivariate normal assumption was violated. Similarly, the potential lack of independence due to multiple isolates per sample did not adversely affect the factor analytic results (but, rather, the increased number of isolates added stability to the factor extraction process).

Factor loadings using the ML method were similar for the transformed and non-transformed data—implying the transformation did not substantially alter inherent patterns in the data. Also, in situations where inferential analyses are not being conducted, it may not be necessary to transform MIC data for use in any of the factor-analysis methods.

5. Conclusions

Factor analysis provides insight into correlations, if they exist, among the MIC patterns for the antimicrobials used in a panel. In this sense, the method can be applied to any panel being used to explore patterns of resistance associated with any bacterial isolates. In addition, when designing future antimicrobial testing studies, a cost-effective alternative might be to consider accounting for the correlations in the data. Antimicrobial-resistance testing is fairly expensive, especially when the number of isolates being tested is large. If certain antimicrobials are strongly correlated, then it might be possible to omit some of the antimicrobials from the panel without substantially reducing the amount of information that is available. Also, as was the case in this study, if there is little variation in the MIC values for a specific antimicrobial, it might not load substantially on any factor. If identification of patterns is the study objective rather than detecting emerging resistance, then it might be useful to omit antimicrobials with little variation in the MIC values from the panel.

Acknowledgements

The study was supported by the USDA, Cooperative State Research, Education, and Extension Service through the Colorado State University—Center for Economically Important Infectious Animal Diseases and by the Research Council of the College of Veterinary Medicine and Biomedical Sciences, Colorado State University. From a thesis to be submitted to the Academic Faculty of Colorado State University in partial fulfillment for the degree of Doctor of Philosophy.

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